

RESEARCH PAPER

Evaluation of Neem (*Azadirachta indica*) Nano-Extracts as Antibacterial Agents Against Uropathogenic Bacterial isolates

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ARTICLE INFO

Article History:

Received 02 May 2026

Accepted 17 July 2026

Published 01 October 2026

Keywords:

Antibacterial

Green synthesis

Nano-Extracts

Silver nanoparticles

UTI

ABSTRACT

Urinary tract infections (UTIs) are considered the second most common type of infection in humans, and a variety of pathogens can cause them, including Gram-positive and Gram-negative bacteria. UTIs are the major health bother that affects millions of people yearly in both a community and hospital setting worldwide. The majority of (UTIs) are caused by Uropathogenic *Escherichia coli*, although other pathogenic bacteria, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, can also cause UTIs. *Azadirachta indica* (neem) is a multipurpose tree with multiple health benefits. It is a well-studied therapeutic plant, known for its diverse phytochemicals and antimicrobial properties against numerous bacterial pathogens. Nanoparticles by means of plant extracts have emerged as an eco-friendly and lucrative approach that leverages phytochemical plummeting agents to produce biocompatible nanostructures with improved antimicrobial activity. These biologically synthesized nanoparticles have been recognized as potent antibacterial agents against a variety of pathogens, including uropathogens, through mechanisms such as membrane disruption and inhibition of biofilm development. Objective: To isolate and identify bacteria responsible for urinary tract infections (UTIs) and evaluate the antibiotic susceptibility patterns of the isolated bacteria, and to assess the potential effectiveness of *Azadirachta indica* (Neem) leaf Nano-Extracts against UTI pathogens. A study was conducted between October 1, 2025, and December 30, 2025. A total of 100 urine specimens from men and women aged 5 to 70 were carefully collected and examined. Bacterial isolates were diagnosed. This was confirmed using HiCrome UTI agar, and the isolated bacteria were tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion method in accordance with CLSI standards. The prepared silver nanoparticles were characterized using two methods, one using an aqueous extract and the other using an alcoholic extract of the neem plant, by several spectroscopic techniques, UV-Vis spectrum, FT-IR spectroscopy, Gas Chromatography GC-MS, TEM, SEM (Scanning Electron Microscopy), and finally with X-Ray (XR-D), which were applied on inoculated plates of Muller-Hinton agar. The disc diffusion method was used to screen the antibacterial activity of both *Azadirachta indica* extract and synthetic antibiotics. Results: The most frequently identified organisms were as follows: 35.9% *Enterococcus faecalis*, followed by 29.3% *Staphylococcus aureus*, 21.0% *E. coli*, and 6.6% *Pseudomonas aeruginosa*, all of which were multidrug-resistant (MDR). The successful synthesis of silver nanoparticles was confirmed by color changes and characterization analyses. UV-Visible spectroscopy showed a peak at 440-460 nm, indicating nanoparticle formation. SEM revealed spherical and uniform nanoparticles. EDAX confirmed the presence of silver. *Azadirachta indica* leaf Nano-Extracts showed significant antibacterial action against every bacterial species at all tested concentrations. *Azadirachta indica* (neem) leaf Nano-Extracts showed strong antibacterial activity against a range of bacterial pathogen strains.

How to cite this article

Ali H., Azeez D., Hamdan I. Evaluation of Neem (*Azadirachta indica*) Nano-Extracts as Antibacterial Agents Against Uropathogenic Bacterial isolates. J Nanostruct, 2026; 16(4):4847-4864. DOI: 10.22052/JNS.2026.04.032

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INTRODUCTION

Urinary tract infections (UTIs) are considered the second most common type of infection in humans, and a variety of pathogens can cause them, including the gram-negative- positive and gram-negative bacteria. UTIs are the major health bother that affects millions of people yearly in both a community and hospital setting worldwide [1]. Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide, affecting millions of individuals annually and imposing a significant public health burden. The majority of UTIs are caused by Uropathogenic *E. coli*, although other pathogenic bacteria such as *K. Pneumoniae*, *P. aeruginosa*, and *E. faecalis* can also be separated from clinical urine samples [2]. Medicinal plant research and applications are expanding every day due to therapeutic phytochemicals, which can stimulate the progress of novel medicines. Most plant-based phytochemicals, e.g., alkaloids, flavonoids, carotenoids, phenolic acids, tannins, saponins, and glucosinolates, have beneficial effects on well-being and avoidance of malignancy. Phytochemicals are secondary aromatic plant metabolites that prevent disease and are extensively present in plants. They are widely recognized for preventing and reducing chronic disease risk (e.g., cancer, cardiovascular, and neurological diseases) and for beneficial mediation in treating these diseases [3]. Amongst these, *A. indica* (neem) is a well-studied. The therapeutic plant is sought for its diverse phytochemicals and antimicrobial properties against numerous bacterial pathogens [4] in India, as this tree does not harm the environment and is free of pests and diseases. *A. indica* is considered one of the most beneficial species for people. This neem tree is commonly referred to as Indian lilac or

margosa. Neem is considered the most diverse and adaptable tree in the tropics, with enormous potential. Neem is a member of the Meliaceae family and is regarded as a botanical relative of mahogany [5].

In recent ages, green synthesis of nanoparticles by means of plant extracts has emerged as an eco-friendly and lucrative approach that leverages phytochemical plummeting agents to produce biocompatible nanostructures with improved antimicrobial activity [6]. These biologically synthesized nanoparticles have been recognized for their potent antibacterial activity against a variety of pathogens, including uropathogens, through mechanisms such as membrane disruption and biofilm inhibition [7]. The aim of this study is to isolate and identify bacteria responsible for urinary tract infections (UTIs), evaluate the antibiotic susceptibility patterns of the isolated bacteria, and assess the potential effectiveness of *A. Indica* (Neem) leaf Nano-Extracts against UTI pathogens.

MATERIALS AND METHODS

The present study was conducted between October 2025 and December 2025 in Iraq Al-Muthanna Maternity and Pediatric Hospital. Written consent was obtained from all the patients participating in this study. This study included a total of 100 patients (46 males and 74 females) suffering from recurrent urinary tract infections at different ages. Patients with well-known risk factors for UTI and vesicoureteral reflux were excluded from the study. All the patients enrolled in the study had not taken any antibiotics for at least three days before the sampling. Selective media are utilized to diagnose different bacterial species. HiCrome UTI agar media facilitates and

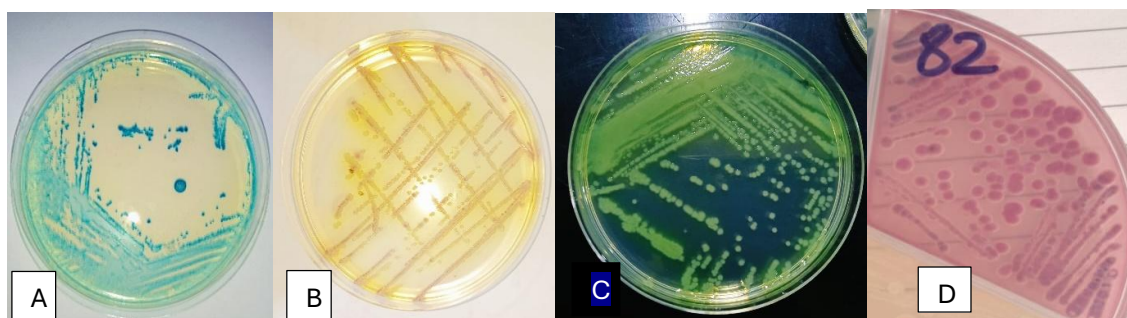


Fig. 1. The bacterial isolates on HiCrome UTI agar A. *E. Faecalis*. B. *S. aureus*. C. *P. aeruginosa*. D. *E. coli*.

accelerates the identification of some gram-positive bacteria and some gram-negative bacteria based on the difference in the shape and color of the colonies. The morphological characteristics of colonies were determined using Gram stain and a light microscope. Muller-Hinton agar was used for antimicrobial sensitivity testing, which was conducted by the agar disc diffusion method. The fresh leaves of the *A. indica* plant were collected from the Fallujah district in Anbar province in September and were classified by a botanist, Dr. Mohamed Baqer Hussein Almosawi, Department of Biology, Al-Muthanna University, and washed with tap water first to remove all darts, then washed with distilled water three times and finally with deionized water, dried it with air. For preparing the two extractors, about 25g of fresh leaves were cut into very small pieces and put in a beaker with 100 mL of deionized water, and the other was extracted with ethyl alcohol. The solution was boiled for 30 minutes. at 70C° in a hot plate.

The extractors were filtered twice after cooling, and the clear extractors were kept at 4C° for further use. To test the antibacterial activity of *A. indica* leaf extract, the aqueous and alcoholic extracts were prepared separately; the aqueous extract was dissolved in sterile deionized water, while the alcoholic extract was dissolved in ethanol. Equal concentrations of both extracts were then prepared 10mg, 15mg, 20mg, 25mg, 30mg, and their antibacterial activity was investigated using the agar etching method.

Biosynthesis of Silver-Nanoparticles

Silver nanoparticles were prepared according to the standard method [4,8]. with some

modifications. Plant extracts contain chemical compounds that facilitate the safe and rapid preparation of nanoparticles by reducing silver ions to the nanoscale. Solutions of silver nitrate with varying concentrations, starting from 5 mg, were prepared by dissolving the silver nitrate in deionized water in a conical flask. The flask was placed on a hot plate with continuous and rapid stirring for 30 minutes. The plant extract was then added dropwise to the solution, monitoring the pH until a color change occurred. The solution was left in a dark place for 24 hours. Using a centrifuge, the nanoparticles were separated and washed three times. The resulting solution was poured into a glass bottle and dried in an oven, yielding a black precipitate. Nanoparticles of the aqueous and alcoholic extracts were prepared using the same method. The resulting precipitate was stored in opaque bottles for further analysis.

Characterization of synthesized Silver-nanoparticles

The prepared silver nanoparticles were characterized using two methods, one using an aqueous extract and the other using an alcoholic extract of the neem plant, by several spectroscopic techniques, Uv-Vis spectrum, FT-IR spectroscopy, Gas Chromatography GC- Mass, TEM, SEM (Scanning Electron Microscopy), and finally, X-Ray (XR-D).

Ethical approval

The principles summarized in the Declaration of Helsinki served as the basis for the conduct of this study. Verbal consent was acquired from the questionnaire form, which was completed by the participants prior to obtaining the samples. The

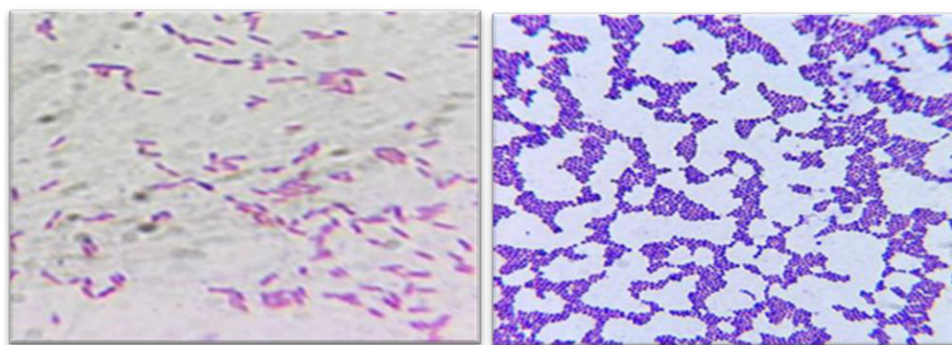


Fig. 2. A) gram-negative B) gram-positive *S. aureus*.

study protocol was examined and approved by a local ethics committee.

Statistical analysis

Statistical analysis was performed using SPSS version 24. Analysis of variance (ANOVA) was used to compare the mean inhibition diameters of bacterial isolates at different concentrations of nano- and aqueous extracts. The chi-square test was used to compare the frequencies and proportions of susceptibility and resistance patterns to the antibiotics and extracts. The results were considered statistically significant at a $p = 0.05$.

RESULTS AND DISCUSSION

Isolation of bacteria associated with urinary tract infections

Based on the results of cultural identification, the majority of isolates were recognized as Gram-positive bacteria in this study. was based on the difference in the shape and color of the colonies on HiCrome UTI agar media, *S. aureus* appeared as golden yellow isolated colonies, *E. faecalis* showed as small blue colonies, *E. coli* appeared as pink to purple, and *P. aeruginosa* was colorless greenish. Regarding bacterial culture growth, the present results showed that 29 (29.0%) of UTI patients

have single bacterial growth and 71 (71.0%) have mixed bacterial infection.

These results showed that 65 out of 181 isolates (35.9%) have *E. faecalis* bacterial infection, followed by 53 isolates (29.3%) have *S. aureus* bacterial infection, 38 isolates (21.0%) have *E. coli* bacterial infection, 13 isolates (7.2%) have *K. pneumoniae* bacterial infection, and only 12 isolates (6.6%) have *P. aeruginosa* bacterial infection. The identification of some gram-positive bacteria and some gram-negative bacteria was based on the difference in the shape and color of the colonies on HiCrome UTI agar media. *S. aureus* appeared as golden yellow isolated colonies, *E. faecalis* showed as small blue colonies, *E. coli* appearance is pink to purple, as shown in Fig. 1, *P. aeruginosa* was colorless and greenish.

Gram stain

This test method is used to detect and distinguish the isolated bacteria based on their color and form. These bacteria are classified as gram-negative bacteria, which have a thin covering of peptidoglycan that appears pink when counterstained with safranin [9]. And gram-positive bacteria, which have a thick peptidoglycan coating that keeps the crystal violets in place and gives them a purple hue [10], as shown in Fig. 2.

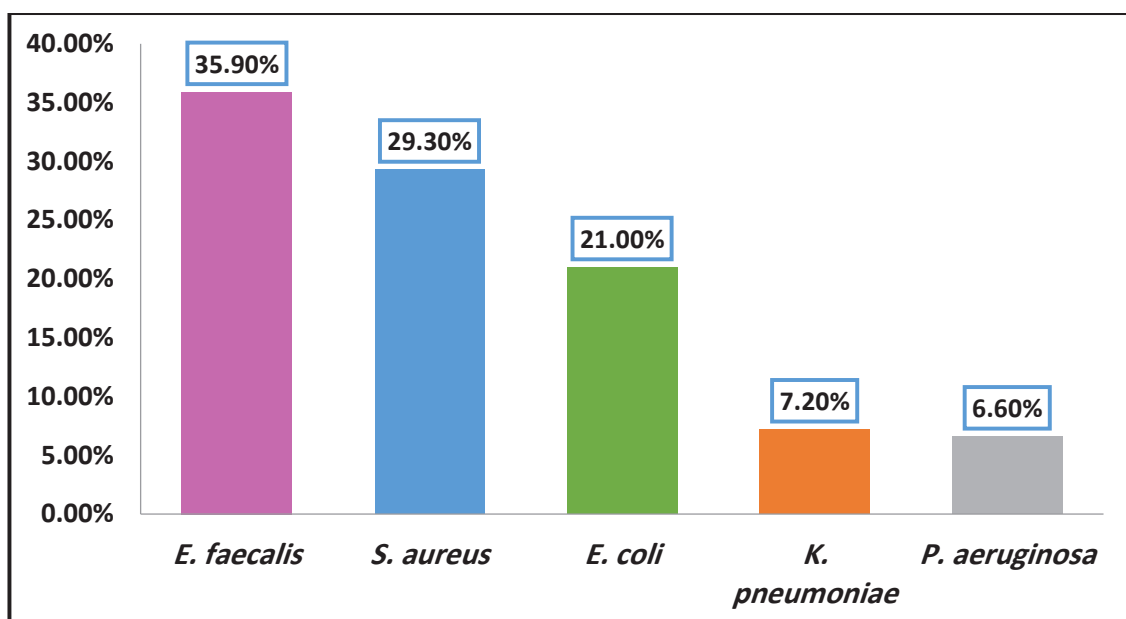


Fig. 3. Percentages of bacterial species of UTI infection.

E. coli is considered one of the main causes of UTIs, infecting about 90% of patients suffering from UTIs in the world [11], when comparing the results of the current study with the previous local, Arabic, and international studies, we find that the incidence of UTIs in the current study is higher than that of the local studies, which was the one conducted by, where the percentage of bacterial isolates isolated from UTIs reached (%39.06). Also, an approach to the study conducted by [12] in hospitals in the city of Baghdad and its surrounding areas, the percentage of bacteria isolated was (%41.6) from urine samples. This difference in infection rates is due to differences in geographical and sanitary conditions, as well as in the number of samples. Taking antibiotics before sampling is also a contributing factor to the high percentage. The result may be due to bacteria adapting to the urinary tract environment and to environmental conditions, not just *E. faecalis*. Suitability, along with clear and powerful harmful factors, increases their ability to cause infection. Another noteworthy finding from the current study is that 53 isolates (29.3%) were from UTI patients with *S. aureus* infection. This result is higher than those found in both [13,14] studies, where the percentages were (8.5%) and (10.52%), respectively.

The increased frequency of *S. aureus* in

this study further underscores the differences observed compared to previous research. Regarding *K. pneumoniae*, the current study found that 13 UTI isolates were due to this bacterium. The percentage in the current study was 7.2%, which is lower than the result reported in a study by [15]. That study showed a 29.2% prevalence of *K. pneumoniae*. It is also lower than [16] Niger. At Delta University Teaching Hospital, the percentage of *K. pneumoniae* isolates in all urine samples reached 30%. The variation in isolation rates may be due to differences in sample collection methods, location, and time of collection, as shown in Fig. 3. These methodological considerations help explain the disparities observed across the studies discussed above.

The ages of the patients ranged from 5 to 70 years, and they were classified into four groups according to their ages. As shown in Table 1, the age groups with the majority of patients were 20-29 years (49.0%). As shown in Fig. 4.

The microbiota of urine changes with age. Changes also occur in microbial communities in other physiological environments [17]. Children and adults have different microbiome communities. These differences result from urinary metabolites, lifestyle changes, personal hygiene, and voiding habits. Adults are found to harbor several bacterial genera across age groups

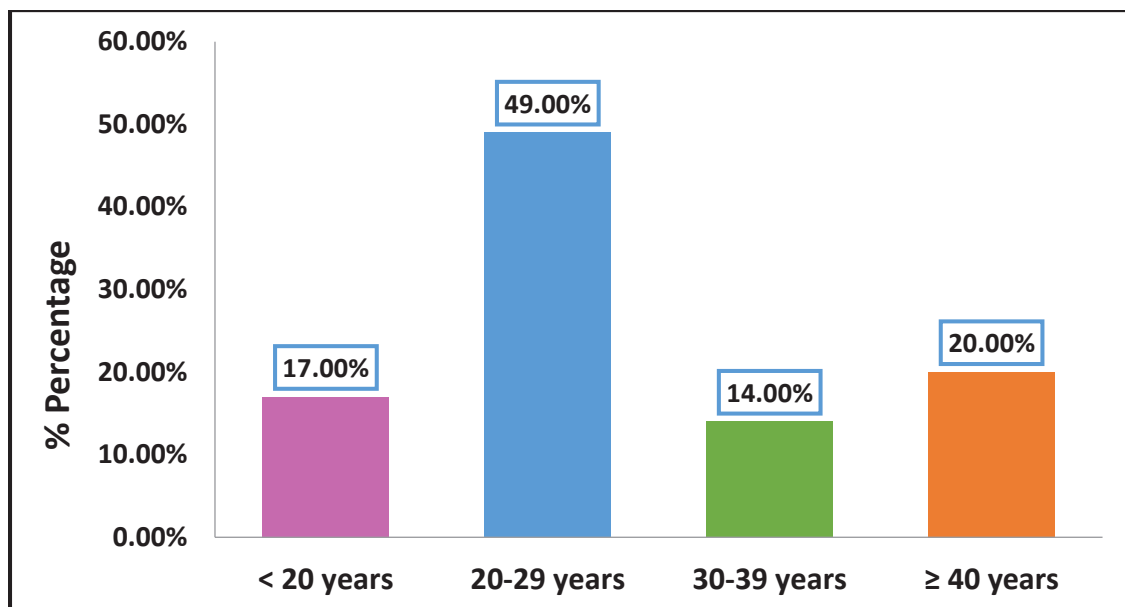


Fig. 4. Age group distribution of patients suffering from UTI infection.

[18]. Research shows that UTIs are more common among middle-aged and older adults than among younger adults. The present findings reveal that younger age groups show less diversity in their bacterial communities. This diversity increases gradually with older age. In this investigation, the most identified organisms were *Enterococcus faecalis*, *Staphylococcus aureus*, and *E. coli* in all age groups. However, *E. coli* was less prevalent among UTI patients under 20 years of age

(Table 1). *E. coli* and *K. pneumoniae* were more often identified in patients aged 20-29 years. *P. aeruginosa* was found only in patients aged 20-29. *K. pneumoniae* was also identified in patients aged 20-29 and 30-39. Changes in bacterial heterogeneity could contribute to the disease's prevalence in women. These results showed that certain microbes were more prevalent in the urinary tract across age groups. Hormonal changes can affect the microbiome. A few studies

Table 1. Distribution of isolated bacteria based on age groups.

Characteristic	No. of isolates	Bacterial species	Percentages	P value
Age groups				
< 20 years, <i>n</i> (%)	23 (12.7%)	<i>E. faecalis</i>	16 (69.7%)	0.001*
		<i>S. aureus</i>	5 (21.7%)	
		<i>E. coli</i>	1 (4.3%)	
		<i>K. pneumoniae</i>	0	
		<i>P. aeruginosa</i>	1 (4.3%)	
20-29 years, <i>n</i> (%)	96 (53.0%)	<i>E. faecalis</i>	26 (27.1%)	0.001*
		<i>S. aureus</i>	29 (30.2%)	
		<i>E. coli</i>	24 (25.0%)	
		<i>K. pneumoniae</i>	6 (6.3%)	
		<i>P. aeruginosa</i>	11 (11.4%)	
30-39 years, <i>n</i> (%)	29 (16.0%)	<i>E. faecalis</i>	10 (34.5%)	0.371
		<i>S. aureus</i>	9 (31.0%)	
		<i>E. coli</i>	6 (20.7%)	
		<i>K. pneumoniae</i>	4 (13.8%)	
		<i>P. aeruginosa</i>	0	
≥ 40 years, <i>n</i> (%)	33 (18.3%)	<i>E. faecalis</i>	13 (39.4%)	0.084
		<i>S. aureus</i>	10 (30.3%)	
		<i>E. coli</i>	7 (21.2%)	
		<i>K. pneumoniae</i>	3 (9.1%)	
		<i>P. aeruginosa</i>	0	

n: number of cases; ¥: Chi-square test; S: significant at *P* <0.05

have found that *Lactobacillus* species increase during puberty and decline after menopause [19]. Younger women who become pregnant may undergo changes in their microbiota. In contrast, those who are not pregnant tend to have more stable microbial compositions [20]. A variety of diseases, such as UTI, have been linked to changes in the urinary microbiome changes over time.

Multiple UTIs in elderly women may lead to a loss of *Lactobacillus* spp. [21]. Recurrent UTIs affect not only the elderly but also young, healthy women [22], potentially altering microbiome diversity, as shown in Table 1.

The present results show that females were more likely to get UTIs than males (69.0% versus 31.0%), as exhibited in Fig. 5. The explanation for

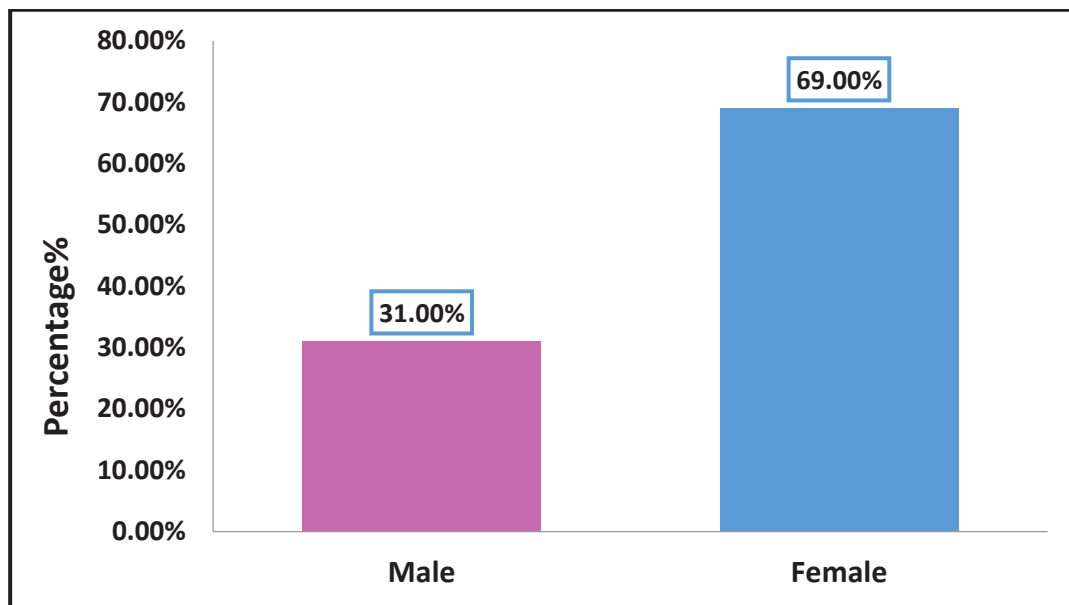


Fig. 5. Gender distribution of patients suffering from UTIs infection.

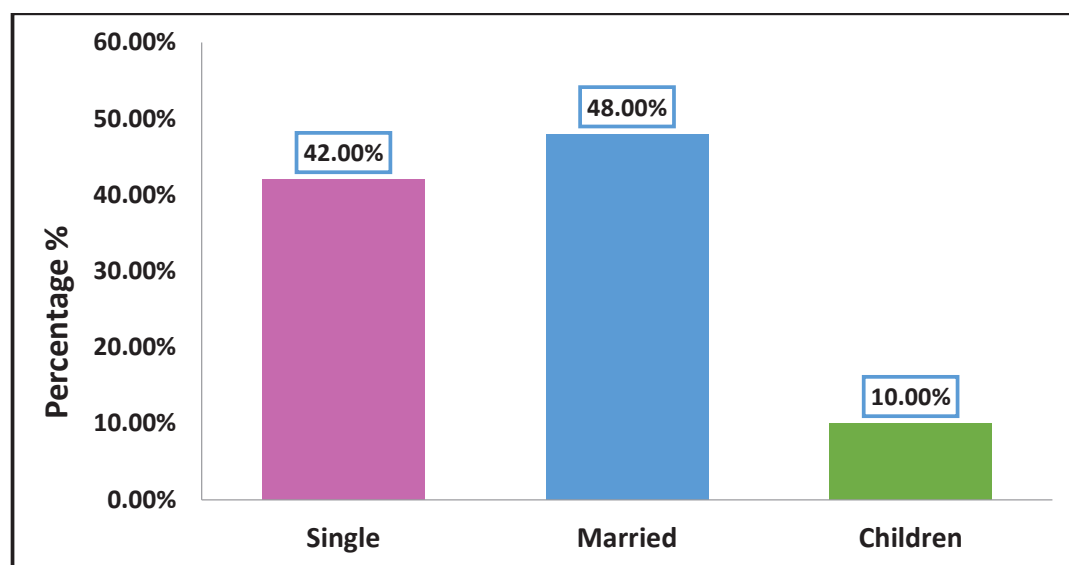


Fig. 6. Frequency distribution of patients suffering from UTIs infection according to social status.

this difference between the incidence of males and females is that women are more susceptible to UTIs, and this reason stems from the fact that the urethra in women is shorter than in men; this means that the bacteria have to travel a shorter distance before reaching the woman's bladder,

which increases her risk of infection [23]. Therefore, based on the current study, it is concluded that UTIs are determined by gender. The researchers explained the difference in these percentages in their study as due to hormonal changes during the menstrual cycle and pregnancy, which affect

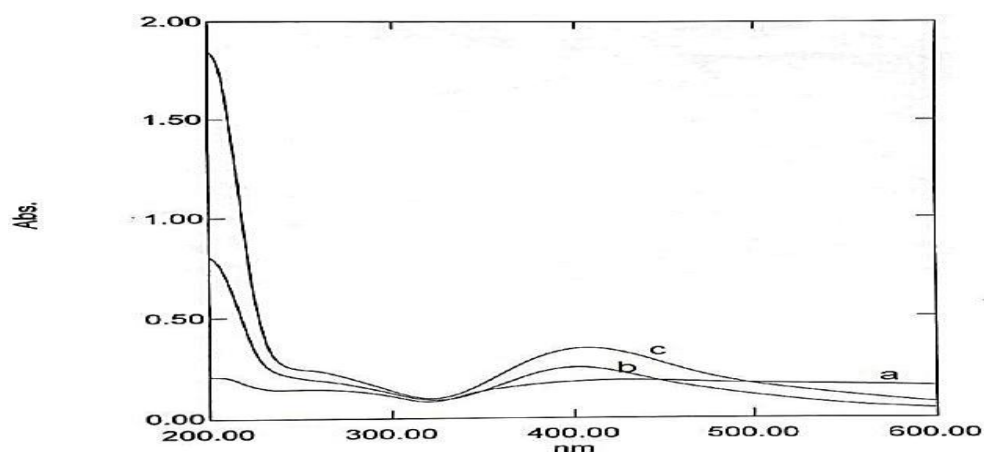


Fig. 7. UV-Vis Absorbance spectra of a) neem extractor, b) synthesized Ag-NPs using aqueous Neem leaves extract, and c) synthesized Ag-NPs using ethanolic Neem leaves extract

Table 2. The frequency distribution UTI patients according to some clinical features.

Characteristic	Patients <i>n</i> (%)
Chronic disease	
Present, <i>n</i> (%)	11 (11.0 %)
Absent, <i>n</i> (%)	89 (89.0 %)
History of UTI	
Single infection, <i>n</i> (%)	10 (10.0%)
Recurrent infection, <i>n</i> (%)	47 (47.0 %)
Absent, <i>n</i> (%)	43 (43.0 %)
Symptoms	
Burning urination, <i>n</i> (%)	52 (52.0%)
lower abdominal pain, <i>n</i> (%)	77 (77.0%)
Without symptoms, <i>n</i> (%)	10 (10.0 %)

n: number of cases.

the acidity of the vagina and urine, potentially enhancing bacterial growth and increasing the risk of infection. During pregnancy, the enlarged uterus puts pressure on the bladder, impeding its complete emptying, which increases the risk of UTI.

Out of 100 patients, 48 (48.0%) are married, 42 (42.0%) are single, and 10 (10.0%) are children, as shown in Fig. 6. Notably, the likelihood of UTI is higher among married patients and may be more than four times that of children.

The frequency distribution of UTI patients by selected clinical features is shown in Table 2. The frequency distribution of UTI patients with chronic disease was as follows: only 11 (11.0%) had chronic disease. The frequency distribution of patients according to history of UTI was as follows: 10 (10.0%) had a single infection, 47 (47.0%) had recurrent infection, and 43 (43.0%) had no previous UTI. The chances of UTI occurrence are higher in patients with recurrent UTI infection, and it can be more than fivefold compared to

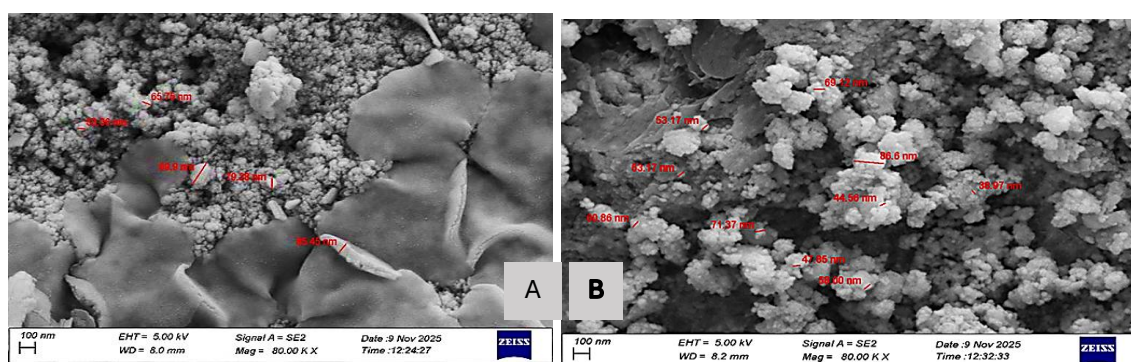


Fig. 8. A) SEM images of aqueous extractor, B) SEM images of alcoholic extractor

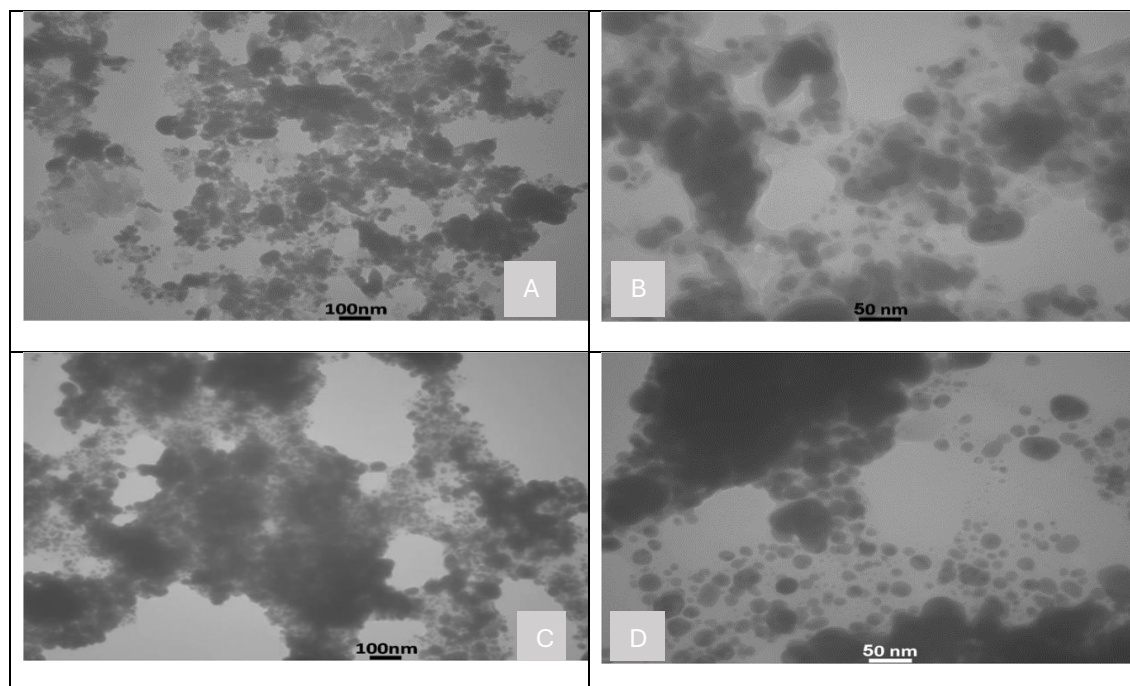


Fig. 9. A,B) TEM images of aqueous extractor, C,D) TEM images of alcoholic extractor

patients with a single UTI infection. Furthermore, the frequency distribution of patients according to symptoms was as follows: 77 (77.0%) patients had lower abdominal pain, 52 (52.0%) had burning urination, and 10 (10.0%).

Biosynthesis is considered one of the best methods used for preparing nanoparticles for this purpose. Not only is it inexpensive, clean, environmentally friendly, and non-toxic, but it is also safe and delivers a high yield of excellent-quality results, which is why we used it in the current project. Silver nanoparticles were prepared using two types of aqueous and alcoholic extracts of the neem plant. Plant extracts have the advantage of containing phytochemicals that aid in reducing silver and converting it into extremely small molecules.

Ultraviolet Analysis (UV-Vis Spectroscopy)

UV-Vis analysis is one of the most important diagnostic tools for confirming the formation

of silver nanoparticles (AgNPs) in liquid media. When neem leaf extract (aqueous and alcoholic) was added to a silver nitrate solution, a gradual change in the solution's color from pale yellow to dark brown was observed, a preliminary physical indicator of the reduction of Ag⁺ ions to Ag. Spectroscopic measurements showed the appearance of a clear absorption band in the range (400–460 nm). This absorption is attributed to surface plasmon resonance, a unique physical phenomenon arising from the collective vibration of conduction electrons at the surface of nanoparticles when exposed to incident light. Comparing the two extracts, the following was observed: The alcoholic extract showed a sharper absorption peak at approximately 422–480 nm, indicating high efficiency in ion reduction and the formation of particles with a narrow size distribution. The aqueous extract showed a relatively wider peak in the 400–490 nm range. As shown in Fig. 7, this may indicate greater

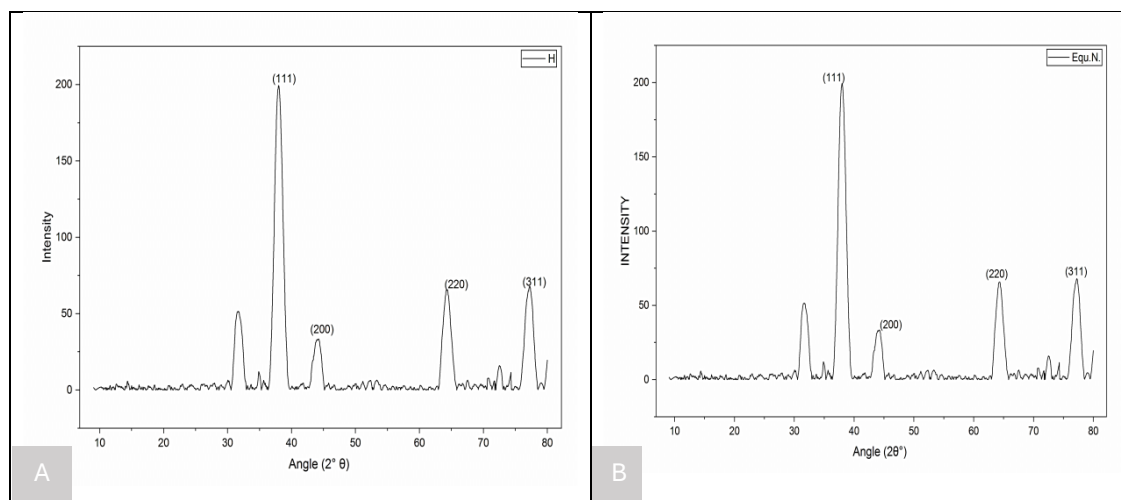


Fig. 10. A) alcoholic extract, B) Aqueous Extract.

Table 3. Properties of Nanoparticles Prepared for Aqueous Extract 1.

Pos. [°2Th.]	FWHM Left [°2Th.]	<i>hkl</i>	D. nm
9.7359	0.1808	-	-
31.6070	0.1808	100	30.78
38.0680	0.9840	111	33.09
44.1478	0.1808	200	65.75
64.1265	0.7872	220	79.25
73.0657	0.1476	222	89.09
77.1906	0.9840	311	85.45

variation in the sizes of the prepared nanoparticles compared to those in the alcoholic medium. The stability of the absorption peak and its lack of shift towards longer wavelengths over time confirms the effective role of phytochemicals in neem (such as nimbin and quercetin) as stabilizing agents that prevent the aggregation of particles. had no symptoms.

FTIR Analysis

The Fourier transform infrared (FT-IR) spectroscopy results of the aqueous extract of neem leaves revealed major absorption bands at 3250–3420 nm, attributed to the stretching vibration of the hydroxyl group (O-H) associated with phenolic compounds and glucosides. A peak at 1635 nm indicated the presence of a carbonyl

group (C=O) or double bonds in aromatic rings. Comparing this spectrum with that of the prepared silver nanoparticles (AgNPs) revealed a shift in these peaks and a decrease in their intensity, confirming the involvement of these active groups in the reduction of silver ions (Ag⁺) to Ag and in their binding to the nanoparticle surface, providing stability and preventing agglomeration.

For the alcoholic extract, the FT-IR spectrum revealed a similar distribution of functional groups, with sharper peaks in the range (2920 and 28501) representing aliphatic (C-H) stretching vibrations, in addition to a strong band at (2890-2799) attributed to the terpene and flavonoid compounds extracted by the alcohol. The marked change in these frequencies after the formation of silver nanoparticles indicates that the nonpolar

Table 4. Characteristics of Nanoparticles Prepared with Alcoholic Extract 2.

Pos. [°2Th.]	FWHM Left [°2Th.]	<i>hkl</i>	D. nm
19.54	0.51	-	-
37.29	0.72	100	38.79
38.19	0.36	111	44.56
64.62	0.98	200	47.85
67.29	1.57	220	53.17
73.17	0.23	222	58
77.16	0.79	311	60.86

Table 5. Antibiotic susceptibility test of different bacterial isolates toward antibiotic.

Abbr	Disc Content	<i>S. aureus</i> N= 53	<i>E. faecalis</i> N= 65	<i>E. coli</i> N=38	<i>P.aeruginosa</i> N=12
AT	Azetronam 30mg	43(81%)	43(66.1%)	36(94%)	9(75%)
CRO	Ceftriaxone 30mg	40(75.4%)	43(66.1%)	35(92.1%)	9(75%)
IPM	Imipenem 10mg	32(66%)	40(61.5%)	24(63.1%)	3(25%)
NA	Nalidixic acid 30mg	35(66%)	40(61.5%)	24(63.1%)	8(66.6%)
AMC	Amoxillin-Clavulanate 30mg	32(66%)	40(61.5%)	30(78.9%)	7(58.3%)
TE	Tetracycline 30mg	45(84.9%)	45(69.2%)	30(78.9%)	7(58.3%)
NX	Norofloxacin 10mg	48(90.5%)	48(73.8%)	28(73%)	2(16.6%)
CIP	Ciprofloxacin 5mg	20(37.7%)	30(46.1%)	8(21%)	2(16.6%)
OF	Olfaxacin 5mg	30(55.5%)	45(69.2%)	24(63.1%)	2(16.6%)
CAZ	Cefotazidime 30mg	30(55.5%)	38(58.4%)	30(78.9%)	8(66.6%)
COT	TrimethoprimSulfamethoxazole25mg	32(66%)	40(61.5%)	18(47%)	2(16.6%)
PIT	Piperacillin-Tazobactam 100/10mg	20(37.7%)	38(58.4%)	8(21%)	8(66.6%)
AK	Amikacin 30mcg	18(33.9%)	40(61.5%)	8(21%)	7(58.3%)
CFS	CefoperazoneSulbactam75/30 mcg	45(84.9%)	45(69.2%)	5(13.1%)	7(58.3%)
MRP	Meropenem 10mg	18(33.9%)	23(35.3%)	5(13.1%)	6(50%)
GEN	Gentamycin 10mcg	20(37.7%)	23(35.3%)	5(13.1%)	6(50%)
CPM	Cefepime 30mcg	30(55.5%)	43(66.1%)	8(21%)	6(50%)
CTX	Cefotaxime 30mg	30(55.5%)	43(66.1%)	8(21%)	7(58.3%)
LE	Levofloxacin 5mg	20(37.7%)	30(46.1%)	8(21%)	2(16.6%)
	P value	0.001**	0.001**	0.001**	0.001**
		¥	¥	¥	¥

¥: Chi-square test; **: significant at P <0.05;

compounds in the alcoholic extract played a pivotal role as reducing and coating agents, explaining the high efficiency and stability observed in the results. Comparing the two extracts, the alcoholic extract shows a higher concentration of compounds (terpenes and flavonoids), reflected in faster nanoparticle formation and greater stability than the aqueous extract. These results demonstrate the success of the green manufacturing method using neem leaves as a safe and environmentally friendly alternative to toxic chemicals.

SEM and TEM Analysis

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to determine the morphological characteristics and size distribution of silver nanoparticles prepared with two neem extracts (aqueous and alcoholic).

Scanning electron microscopy (SEM)

SEM images showed that the nanoparticles predominantly had a spherical shape with a homogeneous surface distribution. Some agglomeration was observed in the samples prepared with the aqueous extract, which may

be attributed to the overlap of large biomolecules in the extract. The alcoholic extract, on the other hand, yielded more dispersed and stable nanoparticles, indicating a higher coverage efficiency of the alcohol-extracted compounds. As shown in Fig. 8.

Transmission electron microscopy (TEM)

To obtain higher resolution and a more detailed understanding of the internal structure, the samples were examined using TEM. Images (3, 4, and 5) confirmed that the nanoparticles were completely separated and encased in a very thin layer of organic material derived from the neem extract, which acts as a physical and chemical stabilizer. Through image analysis and particle diameter calculations, the average particle size was found to be between [insert particle size range here] nanometers. These results are in close agreement with the previously calculated crystalline size from the Scherrer equation in XRD analysis, confirming that the prepared nanoparticles are high-quality single crystals. The consistency between the spherical particle shape in SEM and the fine particle size in TEM supports the hypothesis that the reduction process was

Table 6. Comparison of the antimicrobial activity of the aqueous extract and the nano-aqueous extract against bacterial isolates.

Aqueous extract concentrations	<i>S. aureus</i> N= 53	<i>E. faecalis</i> N= 65	<i>E. coli</i> N=38	<i>P. aeruginosa</i> N=12
10 mg /ml	5mm	5mm	5mm	5mm
15 mg /ml	5mm	10mm	10mm	5mm
20 mg /ml	5mm	10mm	10mm	5mm
25 mg /ml	10mm	15mm	15mm	10mm
30 mg /ml	10mm	15mm	15mm	15mm
P value	0.001* *†	0.001**†	0.00**†	0.001**†

Nano aqueous extract concentrations	<i>S. aureus</i> N= 53	<i>E. faecalis</i> N= 65	<i>E. coli</i> N=38	<i>P. aeruginosa</i> N=12
10 mg /ml	15mm	15mm	15mm	15mm
15 mg /ml	15mm	20mm	20mm	15mm
20 mg /ml	20mm	20mm	25mm	20mm
25 mg /ml	20mm	40mm	25mm	20mm
30 mg /ml	25mm	40mm	30mm	30mm
P value	0.001** †	0.001** †	0.001** †	0.001**†

†: anova; **: significant at P >0.05

controlled by the active components in neem leaves, particularly in the alcoholic medium, which produced smaller and more uniform particles. As shown in Fig. 9.

X-ray Diffraction (XRD) Analysis Results

X-ray diffraction (XRD) analysis was performed to determine the crystal structure and phase of nanoparticles prepared from neem leaf extract. The diffraction pattern showed four distinct main peaks at theta angles of 38.1°, 44.3°, 64.4°, 73°, and 77.4°. These peaks were matched to the (111), (200), (220), and (311) spatial crystal planes, respectively, based on the International Standard Data Card. As exhibited in Fig. 10, these results confirm that the synthesized silver nanoparticles possess a face-centered cubic crystal structure. The appearance of these sharp, narrow peaks clearly indicates the high crystallinity of the

resulting material, and the absence of any additional unexplained peaks reflects the high purity of the prepared nanoparticles and the absence of impurities from the plant extract or silver nitrate used. By measuring the beamwidth at the mid-peak (FWHM) of the most intense peak (level 111), the average crystal size was estimated using the Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

The size was found to be within the nanoscale range [59.59- 42.56 nm], which agrees perfectly with the results obtained from the SEM and TEM analyses. This small size and crystal stability are attributed to the active role of biochemical compounds in neem leaves, which act as a protective coating, preventing the crystals from

Table 7. Comparison of the antimicrobial activity of the alcoholic extract and the nano-alcoholic extract against bacterial isolates.

Alcoholic extract concentrations	<i>S. aureus</i> N= 53	<i>E. faecalis</i> N= 65	<i>E.coli</i> N=38	<i>P.aeru ginosa</i> N=12
10 mg /ml	5mm	10mm	10mm	5mm
15 mg /ml	5mm	10mm	10mm	5mm
20 mg /ml	10mm	15mm	10mm	15mm
25 mg /ml	10mm	20mm	15mm	15mm
30 mg /ml	15mm	20mm	20mm	20mm
P value	0.001** †	0.001** †	0.001** †	0.001** †

Nano Alcoholic extract concentrations	<i>S. aureus</i> N= 53	<i>E. faecalis</i> N= 65	<i>E.coli</i> N=38	<i>P.aeru ginosa</i> N=12
10 mg /ml	10mm	10mm	15mm	5mm
15 mg /ml	20mm	10mm	15mm	15mm
20 mg /ml	20mm	20mm	20mm	20mm
25 mg /ml	40mm	30mm	20mm	20mm
30 mg /ml	40mm	30mm	30mm	20mm
P value	0.001** †	0.001** †	0.001** †	0.001** †

†: anova; **: significant at P <0.05

growing to micron-sized sizes. [24]. The peaks that appeared at the angle θ at 38° belong to (Ag₂O), and the peak at 44° corresponds to (Ag₂O). The Ag/AgO peak at 64° is for the Ag₂O compound, and the most important peak is at 77° , belonging to silver nanoparticles. The high intensity at the 38° peak indicates that the crystals are primarily oriented towards this plane (111), as well as the other planes 200, 220, 311, and 222. Optimal crystal growth occurs at temperatures around 30°C . As shown in Fig. 10.

The peak bandwidth was 38 FWHM (0.98), a significant peak indicating nanoparticle formation. The peak intensity was observed to be relatively higher in the particles prepared with the alcoholic extract, potentially suggesting higher crystallization efficiency compared to the aqueous extract.

Antibiotics susceptibility

Table 5 Antibiotic susceptibility test of different bacterial isolates toward antibiotics. The current study revealed a significant statistical variation ($P < 0.05$) in bacterial susceptibility patterns. *E. coli* isolates showed excellent susceptibility to third-generation cephalosporins such as ceftriaxone (92.1%), consistent with the study by [25], which confirmed the continued efficacy of this class of antibiotics against Enterococcus bacteria in the absence of ESBL strains. *E. faecalis*, on the other hand, exhibited significant susceptibility to norfloxacin (73.8%) and cefepime (66.1%). These results are consistent with the findings of [26,27] in their study on Enterococcus, where they indicated that *E. faecalis* isolates still retained acceptable susceptibility to some fluoroquinolones and cephalosporins compared to the more resistant *E. faecium* strains. In contrast, *P. aeruginosa* exhibited a highly resistant pattern, particularly to ciprofloxacin (16.6%). This finding is in stark contrast to the data from [28], published in Nature Reviews Microbiology, which indicated that global sensitivity to this antibiotic remained above 70%. This suggests high selective pressure in the study environment, leading to the emergence of MDR strains. *S. aureus* demonstrated high sensitivity to tetracycline (84.9%), consistent with [29] report of declining resistance to this antibiotic in certain strains. However, the average response of all isolates to imipenem (61.5%–66%) differs from the study by [30], which identified carbapenems as a strategic option with a sensitivity exceeding 90%.

This raises concerns about the potential spread of carbapenemase enzymes in the tested samples.

The effectiveness of silver nanoparticles and plant extracts (aqueous and alcoholic) against antibiotic-resistant bacteria

The results of the current study showed that neem leaf extracts, both in their crude form and when converted into silver nanoparticles (aqueous and alcoholic), they possess significant antibacterial activity against the tested species. As exhibited in Tables 6 and 7, a substantial improvement in efficacy was observed when transitioning from crude extracts to nanoparticles formulations, with the diameters of the inhibition zones gradually increasing with increasing concentration from 10 to 30 mg/ml. These differences were statistically significant ($P = 0.001$), indicating a direct relationship between the concentration of both crude and nanoparticle extracts and their effectiveness in inhibiting bacterial growth.

While the effect of crude extracts is attributed to their natural content of phytochemicals, the superior effect of nanoparticle extracts is due to the unique physicochemical properties of silver nanoparticles. Their small size and large surface area enable them to interact directly with the bacterial cell wall, disrupting cell membrane permeability and inhibiting metabolic processes within the cell. Furthermore, the nano-formulas demonstrated a higher capacity to generate reactive oxygen species (ROS), which significantly contributes to the destruction of bacterial cellular components compared to crude extracts. The results showed that the traditional aqueous extract of *A. indica* leaves possessed antibacterial activity against all studied isolates. As exhibited in Table 6, although its intensity was limited to moderate. *E. faecalis* recorded the maximum inhibition diameter of 15 mm at the highest concentration, while *S. aureus* recorded 10mm, *E. coli* between 10–15 mm, and *P. aeruginosa* is the least responsive (5–10 mm). These differences can be explained from a physiological and biochemical perspective by the fact that the cellular structure of Gram-negative bacteria, such as *P. aeruginosa* and *E. coli*, contains a complex outer membrane that restricts the entry of active compounds, thus limiting the effect of medium- to large-molecular-weight plant extracts [31].

These results are consistent with those of Owolabi et al. (2025), who reported that

the aqueous extract of neem leaves exhibits antibacterial activity, but is often relatively less potent than organic extracts. Their study recorded zones of inhibition ranging from 10 to 20 mm, depending on the microbial isolate. This consistency is attributed to the fact that aqueous Solvents do not extract active compounds such as phenols and tannins, which may be more soluble in organic solvents, with the same efficiency, thus limiting the concentration of active compounds in the aqueous extract. The current findings also align with the report by [32], who demonstrated that the aqueous extract of neem exhibits antibacterial activity within similar ranges, while methanolic and Ethanol extracts showed higher activity, suggesting that solvent type influences the extraction of active compounds. However, the results of the current study partially differ from those reported by [33], who recorded higher activity of the aqueous extract against certain isolates such as *E.coli* and *K.pneumoniae* (20 mm) using improved extraction methods and advanced filtration techniques. This suggests that the preparation method and the reduction of impurity particles can enhance the efficacy of the aqueous extract. This difference is significant because it highlights how the experimental methodology greatly influences biological results, even when using the same plant. As for the nano-aqueous extract, it demonstrated significantly higher antibacterial activity than the conventional aqueous extract, with inhibition diameters ranging from 15 to 40 mm. As exhibited in Table 6. *E.faecalis* was the most sensitive isolate, with a diameter of approximately 40 mm, followed by *E. coli*, 30 mm, and *S. aureus*, 25 mm, while *P.aeruginosa* was the least sensitive, despite showing improvement compared to the aqueous extract, 20 mm. The increased activity of the nanoparticle aqueous extract can be explained by the fact that nanoparticles have a much larger surface area than conventional molecules, which enhances contact with cell membranes and increases the penetration of active compounds into bacterial cells. Furthermore, they can stimulate the generation of reactive oxygen species (ROS), which damage intracellular structures [34,35]. These results are largely consistent with those reported by [36], who found potent antimicrobial activity of green nanoparticles prepared from neem leaf extract against *E. coli* and *S. aeruginosa* isolates. The study, conducted on *S. aureus*, recorded

inhibition zones of 25 mm, reinforcing the idea that switching to a nano-formula significantly increases antibacterial activity. This finding is further supported by a study by [37], which demonstrated that nano-plant extracts outperform aqueous extracts in their antibacterial activity, attributed to increased surface area and enhanced bioactive pathways at the cell wall. However, the results of the current study differ from some older or less focused nano-drug studies, such as [38], which suggested that the effect of aqueous plant extracts may be limited against highly resistant isolates, and that the improvement in activity was not substantial when producing relatively large particles. This indicates that particle size and precise structure are important determinants of antibacterial activity. A direct comparison between the aqueous extract and the nano-aqueous extract reveals that the nano-extract significantly outperforms the traditional aqueous extract in inhibiting the growth of bacterial isolates. For example, in the case of *E.faecalis*, the maximum Inhibition diameter in the nano-extract is approximately 40 mm, compared to about 15 mm in the traditional aqueous extract. This superiority can be attributed to several factors, including increased contact between microbial particles and nanoparticles, enhanced translocation of active compounds across cell membranes, and the internal oxidative activity resulting from ROS generation. These findings suggest that the nano-transformation of plant extracts offers an effective strategy for enhancing antibacterial activity, particularly against antibiotic-resistant isolates, and represents a promising direction for developing more potent antimicrobial therapies than traditional aqueous extracts. The results of the current study showed that the alcoholic extract of neem leaves possesses varying degrees of antibacterial activity depending on the type of bacteria and the concentration used. As exhibited in Table 7. *S. aureus* exhibited inhibition zones ranging from 5 to 15 mm across different concentrations, indicating moderate sensitivity to the alcoholic extract. *E. coli* showed inhibition zones of 10 to 20 mm at higher concentrations, while *E. faecalis* exhibited inhibition zones of up to 20 mm at the highest concentration. *P. aeruginosa* showed relatively smaller inhibition zones ranging from 5 to 20 mm, indicating a degree of relative resistance compared to the other isolates.

These results indicate that the effectiveness

of the alcoholic extract increases with increasing concentration, a pattern expected in studies relying on active plant compounds. This effect is attributed to neem content of a range of active secondary compounds, such as flavonoids, terpenoids, and limonoids, which have the ability to disrupt bacterial cell membrane integrity and induce intracellular metabolic disturbances, thus inhibiting bacterial growth. These results are consistent with the findings of [39], which demonstrated that plant extracts obtained using organic solvents such as ethanol possess higher antibacterial activity than aqueous extracts, due to their greater ability to extract the active phenolic and terpenoid compounds. These results also align with [40] assertion that neem extracts exhibit clear antibacterial activity against several pathogenic bacteria. However, the results of the current study differ somewhat from those of [41], who reported fewer inhibition zones for neem extracts against *S.aureus* and *E.coli* compared to the findings of the current study. This difference may be attributed to several factors, such as variations in extraction methods, solvent type, concentrations tested, or even bacterial strains used in the experiments, all of which play a significant role in determining antibacterial activity. The reduced sensitivity of *P.aeruginosa* in this study is consistent with [42] finding that Gram-negative bacteria possess additional resistance mechanisms that reduce the penetration of antibacterial compounds into the cell. Comparing the results of the conventional alcoholic extract with the alcoholic nano-extract clearly demonstrates a significant increase in antibacterial activity in the nano-formulation. *S. aureus* exhibited the largest inhibition zones, reaching approximately 40 mm at the highest

concentration, while *E.coli* and *E. faecalis* showed inhibition zones of approximately 30 mm, and *P.aeruginosa* showed approximately 20mm. These results are consistent with [43] study, which indicated that nanoparticles synthesized using plant extracts that possess higher antibacterial activity than conventional extracts due to the increased surface area of the nanoparticles and their superior ability to interact with microbial cells. These results also align with [44] findings, which demonstrated that plant nanoparticles possess a high capacity to penetrate bacterial cell walls and disrupt the cell membrane permeability, in addition to their ability to produce reactive oxygen species that damage proteins and nucleic

acids within bacterial cells. However, *P.aeruginosa* showed less sensitivity even at the nanoscale level, consistent with [45] assertion that this bacterium possesses advanced resistance mechanisms, such as multidrug excretion pumps and the ability to form biofilms, making it more resistant to many antibacterial agents compared to other bacterial species.

CONCLUSION

This study showed that bacteria from individuals with UIT infections, with varying patterns of antibiotic susceptibility, can be successfully isolated and identified. The study has effectively demonstrated the potential of neem extracts for environmentally friendly production of silver nanoparticles (AgNPs). These plant-mediated AgNPs have proven to be a viable alternative to traditional antibiotics, with exceptional antibacterial activity against a wide range of UTI infections. The produced AgNPs exhibit remarkable stability, as well as antibacterial properties, making them suitable for a range of biomedical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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